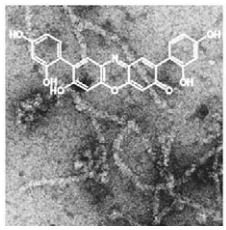
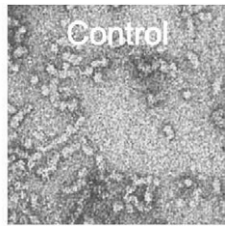
amyloid fibrils cause neuronal dysfunction in neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. Biochemical and cell biological studies indicate that amyloid formation pathways can be manipulated with small molecules. This suggests that stimulation of amyloid polymerization with small molecules might reduce the prevalence of transient, toxic aggregation intermediates. We have recently demonstrated the acceleration of alpha-synuclein and Abeta fibrillogenesis through the action of the orcein-related small molecule, which leads to a decrease in toxicity neuronal cell models.





These results support the hypothesis that small, diffusible pre-fibrillar amyloid species rather than mature fibrillar aggregates are toxic for mammalian cells. They also suggest that compound-mediated acceleration of amyloidogenesis might be a promising therapeutic strategy for amyloid diseases.

2314-Pos Board B84

Alzheimer's Disease at 30? Is that Possible?

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Amyloid diseases, such as Alzheimer's disease, are neurodegenerative disorders that have been introduced by protein misfoldings into amyloid fibrils. The Amyloid Precursor Protein irregularly cleaves the β-amyloid (Aβ) peptide, causing protein misfoldings to aggregate to form the hallmark plaques. For both Aβ(1-40) and Aβ(1-42), a tendency of fibril formation has demonstrated to self-assemble from a non-toxic monomer state to a lethal fibrillar state. Prior research focused only on the 16-21 region of the peptide, however, it is equally important to examine the hair-pin region without the presence of residues 16-21. In order to study the effect on fibril formation without this ''KLVFFA'' region, the 22-35 sequence was chosen . The Italian (E22K) and Arctic (E22G) point mutations lead to changes in time of fibril formation as well as solubility and toxicity of fibrils. The single-point mutations are believed to promote early onset of AD compared to the wild type (WT), prematurely producing clinical and neuropathological features which are unchanged from those of late onset AD. The use of Attenuated Total Reflection Infrared Spectroscopy, ATR-IR, and Ultraviolet Visible Spectroscopy, UV-Vis, on the 22-35 sequence confirmed the formation of structures synonymous with toxic beta sheets. Using Congo Red dye, which binds pentamerically to beta sheet fibrils, secondary structures have been confirmed.

2315-Pos Board B85

Peptides - Beta-Sheet Folding from Hairpins to Aggregates

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Selected examples of turn sequence and hydrophobic contact stabilized β-hairpin peptides were previously studied using ECD, fluorescence, IR and VCD spectroscopies to assess stability of β-hairpin formation. Extending this, two three-stranded β-sheet peptides, based on modified Trpzip sequences, using D-Pro-Gly (B3pG) or Asn-Gly (B3GN) turn sequences gave ECD spectra reflecting cross-strand Trp-Tyr aromatic interactions, and indicated both were partially multi-stranded. Both showed initial IR spectra at low temperatures indicative of extended β-sheet structure that were more characteristic of an aggregate than a small oligomer structure. Thermal variation of their IR spectra gave strikingly different behaviors. B3pG reversibly unfolded from aggregated β-like structure at low temperature to disordered at high temperature. B3GN formed aggregates at low temperature, became disordered with heating, but upon re-cooling gave typical soluble β-sheet peptide spectra, which was could be reversibly unfolded. ThT binding to dilute B3GN, but not B3pG, caused a fluorescence enhancement, consistent with fibril formation. These results suggest that turn sequence mutation leads to different micro- and macro-structures, resulting in tuning their structurally related properties. Modifying the sequences to reduce hydrophobicity (aromatic residues) but incorporate Aib-Gly turns gave partially folded peptides with reversible folding but less stability. Taking another view of β-sheet based peptide aggregation, we studied fibril formation in glutamic acid oligomers at low pH (β2 form). We had shown that poly-Glu IR is relatively insensitive to mixing of D and L isomers, which causes a loss of long range order (fibrilization), as seen in EM, but VCD is hypersentive to this change, providing a new way of detection of long range ordering (chirality) in fibril formation as opposed to aggregate forms. The oligo-Glu peptide models add isotopic labeling to provide new insight into the structure of the fibrils formed.

2316-Pos Board B86

Exploring the Effect of Mutations on the Conformational Landscape of Amyloidogenic Antibody Fragments

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Antibodies are composed of heavy chains and light chains. In Light Chain Amyloidosis (AL), antibody-secreting cells export only the light chains. These light chains are prone to misfold, forming amyloid fibers, which get deposited in various organs, leading to organ disfunction and patient death. The transition from the native state to the amyloid fiber requires partial or total denaturation. The structures of monomeric precursors to the oligomeric nuclei, or of those assembled at the ends of the fibers, are unknown. There is an inverse correlation between the stability of the native state and the speed of fiber formation, suggesting that lower stability allows the population of ''excited states'' of the native ensemble, some of which could be amyloid fiber precursors. Starting from the crystal structure of a variable light chain domain belonging to class 6a (one of the most common in clinical cases of AL), we generated point mutants that eliminate charges (R24G and D52A) or a proline (P7S). These mutants destabilize the native state, and speed up fiber formation (for R24G and P7S). We carried out MD simulations at three temperatures (298, 398 and 498K), to explore the effect of these mutations on the conformational landscape. We found many metastable unfolding intermediates, which have eluded experimental detection because their fluorescence is indistinguishable from that of the native state. A common early unfolding intermediate exposes strand D, which has a high potential for fiber formation according to ZipperDB. Those variants with a higher speed of fiber formation expose this area with greater frequency.
We are thankful for computer resources at: Centro Nacional de Supercómputo, IPICyT; Kan Balam, UNAM; Sputnik, IBT-UNAM; Orion, FC and CIQ-UAEM.
Funding: CONACYT 102182 and 133294.

2317-Pos Board B87

Monomeric Amyloid β Proteins in Reverse Micelles are in Folded β Sheet Structure

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Amyloid beta (Aβ) proteins aggregate to form insoluble fibrils in the brains of persons with Alzheimer's disease (AD). X-ray diffraction studies reveal cross-β structure in these fibrils, while solid state NMR studies indicate that this structure consists of in-register parallel β-sheets. Proteins with these characteristics accumulate in over a dozen different diseases, which have come to be known as protein misfolding diseases. The trigger that induces the misfolding of Aβ proteins in AD is unknown, and neither is the mechanism by which monomeric Aβ proteins add to the growing end of a fibril. In order to characterize the structure of Aβ proteins under conditions in which they are monomeric but not aggregated, we have encapsulated the 40-residue form of the Aβ protein (Aβ40) into reverse micelles formed from sodium bis (2-ethylhexyl) sulfosuccinate (AOT) and examined them with transmission FTIR spectroscopy. Several types of evidence indicate that the encapsulated proteins are monomeric, yet the spectra suggest that they have β-sheet secondary structure. The spectra of a polypeptide with the same amino acid composition as Aβ40 but with a scrambled sequence show a random-coil structure. These results suggest that Aβ40 is capable of forming both antiparallel β structures in a reverse micelle, and parallel β structure in an amyloid fibril. We speculate that antiparallel β structure may help pre-organize the protein for adding onto the end of a growing fibril.

2318-Pos Board B88

Eating and Sleeping, Is this a Cure for Alzheimer's Disease?

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The accumulation of misfolded amyloid proteins has proven to be the common link between neurodegenerative diseases such as Alzheimer's, Huntington's, and Parkinson's disease. According to previous studies done by numerous